Characterizing the protonation states of the catalytic residues in apo and substrate-bound human T-cell leukemia virus type 1 protease

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A B S T R A C T

Human T-cell leukemia virus type 1 (HTLV-1) protease is an attractive target when developing inhibitors to treat HTLV-1 associated diseases. To study the catalytic mechanism and design novel HTLV-1 protease inhibitors, the protonation states of the two catalytic aspartic acid residues must be determined. Free energy simulations have been conducted to study the proton transfer reaction between the catalytic residues of HTLV-1 protease using a combined quantum mechanical and molecular mechanical (QM/MM) molecular dynamics simulation. The free energy profiles for the reaction in the apo-enzyme and in an enzyme – substrate complex have been obtained. In the apo-enzyme, the two catalytic residues are chemically equivalent and are expected to be both unprotonated. Upon substrate binding, the catalytic residues of HTLV-1 protease evolve to a singly protonated state, in which the OD1 of Asp32 is protonated and forms a hydrogen bond with the OD1 of Asp32′, which is unprotonated. The HTLV-1 protease–substrate complex structure obtained from this simulation can serve as the Michaelis complex structure for further mechanistic studies of HTLV-1 protease while providing a receptor structure with the correct protonation states for the active site residues toward the design of novel HTLV-1 protease inhibitors through virtual screening.

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1. Introduction

Human T-cell leukemia virus type 1 (HTLV-1) protease cleaves a viral polyprotein precursor to form individual mature proteins during the life cycle of human T-cell leukemia virus type 1 (Martins et al., 2012), which has been convincingly associated with adult T-cell leukemia (ATL) (Poiesz et al., 1980; Yoshida et al., 1984), tropical spastic paraparesis/HTLV-1 associated myelopathy (TSP/HAM) (Gessain, 1996), and numerous chronic diseases, including inflammation of the eye (uveitis) (Mochizuki et al., 1992), joints (arthritis) (Abe et al., 2004), muscles (myositis) (Inose et al., 1992), and skin (dermatitis) (McGill et al., 2012). Currently, no effective therapies are available for these HTLV-1 associated diseases. HTLV-1 protease is an attractive target for the development of inhibitors that treat HTLV-1 associated diseases due to its critical roles for HTLV-1 virion replication (Tözsér and Weber, 2007). Understanding the catalytic mechanism of HTLV-1 protease is essential when designing improved inhibitors for this enzyme.

Like other retroviral proteases (Wlodawer and Gustchina, 2000), HTLV-1 protease is a homodimeric aspartic protease with two catalytic aspartic acid (Asp) residues from each subunit in its active site. The catalytic mechanism of HTLV-1 protease remains poorly understood at the atomic level. The catalytic process of aspartic proteases may involve a general acid-base mechanism; specifically, one aspartic residue at the active site is protonated to serve as a general acid, while the other remains unprotonated to serve as a general base (Suguna et al., 1987; Hyland et al., 1991; Dunn, 2002; Brik and Wong, 2003). Although the enzymatic properties of HTLV-1 protease have been thoroughly studied (Louis et al., 1999; Kadas et al., 2004) and molecular dynamics simulations have been performed on both the apo and ligand-bound forms of HTLV-1 protease (Rucker et al., 2011), a detailed study of the protonation states of the catalytic residues (Asp32 and Asp32′) in HTLV-1 protease have not been reported; these data are important for understanding the catalytic mechanism and designing novel inhibitors for HTLV-1 protease. Correspondingly, the protonation states of the active site Asp residues of the homologous HIV-1 protease have been widely studied (Smith et al., 1996; Piana and Carloni, 2000; Piana et al., 2001; Wang et al., 1996; Czodrowski et al., 2007; Torbeev and Kent, 2012). Smith et al. (1996) used ^{13}C NMR experiments to characterize the protonation

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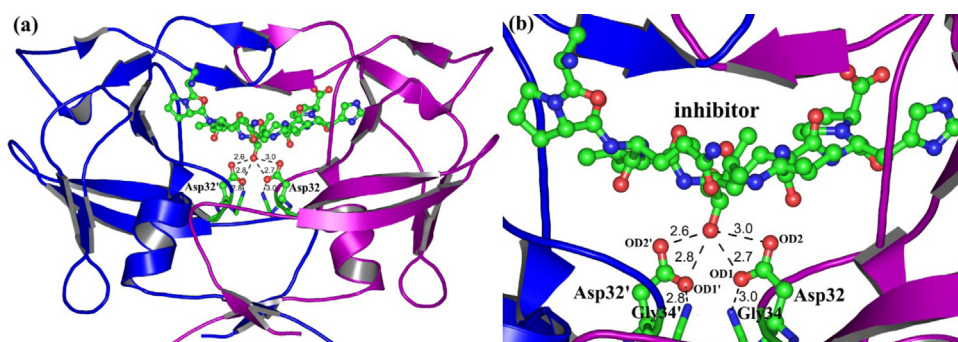E-mail address: sma@towson.edu (S. Ma).

Fig. 1. (a) The complex structure of HTLV-1 protease (subunits A (purple) and B (blue)) and a statine-containing peptide inhibitor (subunit I, ball and stick) (Satoh et al., 2010), the two active site aspartic residues (Asp32 from subunit A and Asp32′ from subunit B) are labeled. (b) Magnified view of the active site showing the interactions between the hydroxyl oxygen on the inhibitor and the carboxylic oxygen atoms (OD1 and OD2) of Asp32 and Asp32′, as well as the interactions between the OD1 of Asp32 and Asp32′ and the N of Gly34 and Gly34′, respectively. Atom names of Asp32′ are labeled with a prime. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

states of the active site Asp residues in the apo and pepstatin-bound HIV-1 protease. They concluded that in the apo enzyme, both Asp residues are deprotonated; in the HIV-1 protease–pepstatin complex, one Asp is protonated and the other is not. Wang et al. (1996) performed solution state NMR studies on a complex of HIV-1 protease with inhibitor KNI-272. They also reported that one Asp is protonated in the active site while the other is not in this enzyme–inhibitor complex. During a recent ^{13}C labeled NMR study, Torbeev and Kent (2012) reported that one active site Asp is protonated and the other is unprotonated in the apo HIV-1 protease, and the same conclusion was also reached by Piana and Carloni (2000), who performed an ab initio study on the apo form of HIV-1 protease. Piana et al. (2001) carried out ab initio molecular dynamics simulations on simplified models of HIV-1 protease–pepstatin complex, suggesting that both of the active site Asp residues are protonated in this enzyme–inhibitor complex. After performing pK_a calculations on a complex of HIV-1 protease with a pyrrolidine containing inhibitor, Czodrowski et al. (2007) reported that both catalytic Asp residues are deprotonated in this complex. Evidently, the protonation states of the catalytic Asp residues remain uncertain for the apo and ligand-bound forms of HIV-1 protease; therefore, additional studies are required to understand the protonation states of the active site residues in HIV-1 and HTLV-1 protease. In this work, combined quantum mechanical and molecular mechanical (QM/MM) (Warshel and Levitt, 1976; Field et al., 1990; Gao and Xia, 1992) molecular dynamics (MD) simulations have been performed to determine the protonation states of the two catalytic aspartic acid residues in the apo and substrate-bound HTLV-1 protease.

The X-ray crystal structures available for HTLV-1 protease are all hexamers with three active sites (Li et al., 2005; Satoh et al., 2010). Because the active form of HTLV-1 protease is a homodimer, we focused on one of the three active sites during our simulations. The structure of HTLV-1 protease in complex with a statine-containing peptide inhibitor (PDB ID: 3LIY) (Satoh et al., 2010) and its A, B, and I subunits have been used during our simulations, where 'A' and 'B' are the two subunits of HTLV-1 protease (Asp32 is from subunit A and Asp32′ is from subunit B. The atom names and residues from subunit B are labeled with a prime throughout this paper), and 'I' represents the inhibitor (Fig. 1).

Fig. 1(b) shows that when protonated, any one of the four carboxylic oxygen atoms in Asp32 and Asp32′ can form a strong hydrogen bond with the inhibitor. And the OD1 of Asp32 and Asp32′ form hydrogen bonds with the N of Gly34 and Gly34′, respectively. Therefore, the protonation states of the two active site residues Asp32 and Asp32′ cannot be determined from the X-ray

crystal structure. To characterize the protonation states of Asp32 and Asp32′ during catalysis, we constructed a complex structure of HTLV-1 protease with a substrate, then undertook combined QM/MM molecular dynamics simulations on the complex structure. Our simulations demonstrated that the enzyme–substrate complex structures with OD2 protonated in both Asp32 and Asp32′ led to the CB—CG bond rotation; therefore, the enzyme–substrate complex structures converged to a state where the OD1 atom from either Asp32 or Asp32′ is protonated. To further determine whether the OD1 atom of Asp32 or Asp32′ is protonated in the complex structure of HTLV-1 protease and its substrate, we carried out free energy simulations for the proton transfer reaction between Asp32 and Asp32′. In addition, we performed free energy simulations for the same proton transfer reaction in the apo HTLV-1 protease, to understand the effects of substrate binding on the protonation states of the active site aspartic acid residues.

2. Computational details

2.1. The structure of the apo and substrate-bound HTLV-1 protease

All of the available X-ray crystal structures of HTLV-1 protease are enzyme–inhibitor complexes. Our simulation began with one of these structures (PDB ID: 3LIY) (Satoh et al., 2010), which is a hexamer with three active sites. Because the active form of HTLV-1 protease is a homodimer, we focused on one of the three active sites. Fig. 1(a) shows a homodimer of the HTLV-1 protease in complex with a substrate-analog inhibitor (Ace-Ala-Pro-Gln-Val-Sta-Val-Met-His-Pro where 'Sta' represents statine) bound to the active site. The A, B and I subunits from the original structure (3LIY) were used in our simulations, where 'A' and 'B' are the two subunits of the HTLV-1 protease, and 'I' represents the inhibitor. To simulate the proton transfer reaction between Asp32 and Asp32′ in the apo HTLV-1 protease, we removed the inhibitor from the active site and equilibrated the structure in solvent before undertaking the free energy simulations.

Studies comparing the substrate specificity of HTLV-1 protease have indicated that a substrate with a sequence of Lys-Gly-Pro-Pro-Val-Ile-LEU–PRO-Ile-Gln-Ala-Pro showed the highest specificity (Louis et al., 1999; Kadas et al., 2004). Therefore, during our simulations, we decided to choose a shorter substrate (Ace-Pro-Val-Ile-LEU-|-PRO-Ile-NMe) that includes the same LEU-PRO cleavage site, with the N- and C-termini capped by an acyl (Ace) group and an N-methyl amino (NMe) group, respectively. This substrate is long enough to include the cleavage site between the